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STEROLS AND A GLYCOSIDE FROM THE FLOWERS OF INULA GRANTIOIDES

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ABSTRACT

The flowers of *Inula grantioides* were analysed for their sterol composition. Chromatographic separation of the acetone extract of the plant material resulted in the isolation of β-sitosterol, stigmasterol and β-sitosteryl glucoside. 11-Hentriacontene, pentatriacontene and a saturated hydrocarbon, pentatriacontane, were also obtained for the first time from the flower of the plant.

Introduction

In the course of systematic studies on individual parts of the plant, *Inula grantioides* (Compositae) we have earlier reported (Burdi *et al*, 1990) the fatty acid constituents obtained from its flowers. The present work describes the isolation of β sistosterol, stigmasterol and a steriodal glycoside, β-silosteryl glucoside alongwith three hydrocarbons from the flowers of the plant.

Previous workers have reported (Harborne, 1977; Sethi et al, 1979; Singha, 1983) the sterols from different species of the genus Inula. Though only β-sitosterol has been reported earlier (Bhutto, 1974; Ahmad, 1990) from the plant as a whole, it has been isolated for the first time from its flowers. However, stigmasterol and β-silosteryl glucoside have not yet been reported from this plant. Hence *Inula grantioides* is found to be a new source regarding their isolation. Literature survey revealed that the hydrocarbons, 11-heotriacontene, pentatriacontene and pentatriacontane have also been obtained for the first time from this plant.

Materials and Methods

Plant material and extraction

The flowers of *Inula grantioides* were collected from Jamshoro (Sindh), Pakistan, during the spring season, dried under shade, and crushed to a coarse powder and extracted exhaustively with acetone at room temperature. The solvent was evaporated from the extract under reduced pressure to afford a thick brownish viscous material (67g).

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Chromatographic separation

Acetone extract of the plant material was elated on a column of silica gel with n-hexane using ethyl acetate as gradient. Fraction B(3.38g) obtained by eluting the extract with hexane-ethyl acetate (4:1, vh) was rechromatogaphed on a silica column using a mixture of hexane and ethyl acetate as eluent system in various ratios. A subtraction (0.62g) eluted with hexane-ethyl acetate (9:1, v/v) was treated with hexane (cold) to give insolube mass (O.08g) which was recrystallized from hexane (warm) to give β-sitosterol (1) and stigmasterol (2) in the form of a homogenous mixture (0.01g). These compounds were obtained also from the latter fractions.

The glycoside, β-sitosteryl glucoside (3) was obtained by eluting the acetone extract on column with ethyl acetate-methanol (3:2, v/v). The fraction obtained in this system was separated into an oily (11.62g) and a solid (1.07g) material. The latter portion was rechromatographed on column by medium pressure (flash) chromatography. The subtraction obtained from this column by chloroform-methanol (19:1, v/v) was again chromatographed on LOBAR column (E. Merck) to get β-sitosteryl-)3-D-glucoside (0.022g) using chloroform-methanol (49:1, v/v) as mobile phase.

The earlier fraction (A) of acetone extract yielded 11-hentriacontene (4), pentatriacontene (5) and pentatriacontane (6) when eluted with hexane-ethyl acetate (9:1, v/v).

Results and Discussion

The identification of sterols, the steroidal glycoside, and the hydrocarbons obtained from the flowers *of Inula grantioides* was made by direct comparison with spectroscopic data i.e. ^{1}H-NMR, ^{13}C-NMR and MS.

Compounds (1) and (2) were obtained in the form of homogeneous mixture which could not be separated by repeated chromatographic and crystallization techniques. ^{13}C-NMR spectrum proved to be very useful in distinguishing the two sterol components. The spectrum showed sufficient number of peaks giving the evidence of two compounds. Twenty seven peaks of each component correspond to those reported (Holland *et al*, 1978) for the basic skeleton of stigmast-5-ene. The predominant difference appeared regarding the values (Table 1) of C-22 and C-23 positions which were recorded at 6 34.01 and 6 26.19 respectively for 1, and at 6 138.28 and 6 129.31 respectively for 2. Similarly, ^{1}H-NMR data supports their proposed structures. The values recorded for 1 and 2 were coinciding with those reported (Jain and Banks, 1968; Rubinstein *et al*, 1976) for β-sitosterol and stigmasterol. Mass values, alongwith fragmentation pattern, were also found in conformity with those published (Jain and Banks, 1968; Lenfant *et al.*, 1970) in the literature.

Compound (3) was identified through comparison of the data published (Kati, 1987; and Sakakibara *et al*, 1983) for β-sitosteryl-β-D-glucoside. The presence of sugar moiety in (3) was indicated by ^{1}H-NMR spectrum. It showed one anomeric proton

doublet at b 4.25 (1=7.6 Hz). The coupling constant indicating that the hexose unit is present in β-anomeric form.

Table 1
^{13}C-NMR chemical shits of compounds (1) and (2).

	δ (ppm)				
Position	1	2	Position	1	2
1	37.30	37.30	16	28.27	28.92
2	31.73	31.73	17	56.12	56.02
3	71.64	71.64	18	11.90	12.26
4	42.36	42.36	19	19.42	19.42
5	140.77	140.77	20	36.16	48.48
6	121.70	121.70	21	19.08	21.09
7	31.73	31.73	22	34.01	138.28
8	31.96	31.96	23	26.19	12931
9	50.20	50.20	24	45.91	5127
10	36.55	36.55	25	29.24	31.91
11	21.13	21.13	26	18.82	19.02
12	39.83	39.73	27	19.84	21.24
13	42.26	42.26	28	23.13	25.42
14	56.82	56.82	29	12.01	12.09
15	24.33	2433	-	-	-

The ^{13}C-NMR spectrum of (3) showed 35 carbon signals out of these 29 were due to the aglycone (table 2). The downfield shift of C-3 as compared to the values of relevant carbon of β-sitosterolindicates the sugar linkage at that position. The chemical shifts of the sugar unit (table 2) closely resemble those observed in the literature (Sakakibora et al, 1983; Gorin and Mazurek, 1975) for glucose.

The mass spectrum of aglycone moiety exhibited a very weak molecular ion peak at m/z 414 (M^+, $C_{29}H_{50}O$). A prominent perk appeared at m/z 329 which is characteristic for sterols with C5-C6 double bond. Other peaks were also found in conformity with those reported (Jain and banks, 1968; Lenfant *et al.*, 1970) for β-sitosterol. Hence the structure of (3) is elucidated as 3-O-β-D- glucopyranosyl-3-β-sitosterol.

In addition to the above mentioned compounds, three hydrocarbons (4), (5), and (6) were isolated for the first time from this plant and identified through comparison of

the spectral data (Helles and Milne, 1978). The molecular formulae were established as $C_{31}H_{62}$, $C_{35}H_{70}$ and $C_{35}H_{72}$ respectively by the high resolution mass spectral analysis which showed the molecular ion peaks at ma 434.4857, 490.5482 and 492.5644 respectively for (4), (5) and (6).

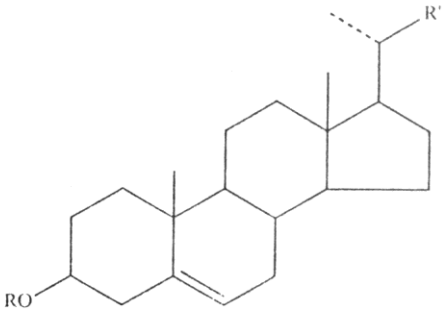
Table 2
^{13}C-NMR chemical shifts of compound (3)

Position	δ (ppm)	Position	δ (ppm)
Aglycone moiety		18	11.16
1	36.83	19	18.16
2	29.10	20	35.65
3	78.58	21	18.10
4	39.35	22	3351
5	139.99	23	25.69
6	121.47	24	45.50
7	31.43	25	28.76
8	31.48	26	18.30
9	49.65	27	19.01
10	36.64	28	22.60
11	2058	29	11.16
12	38.19	*Sugar moiety*	
13	41.7	1'	100.74
14	56.36	2'	73.22
15	23.74	3'	75.61
16	27.69	4'	69.95
17	55.67	5'	76.19
		6'	61.36

Spectral data

1: ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.67 (3H, s, H-18), 0.79 (311, d, = 7 Hz, H-27), 0.83 (3H, t,J = 7Hz;H-29), 0.84 (3H, d, J = 7Hz, H-26), 0.91 (3H, d, J = 6.5 Hz, H-21), 1.00 (3H, s, H-19), 3.52 (1H, m, H-3), 5.34 (1H, br. m, H-6); ^{13}C-NMR (see Table 1); m/z 414 (M^+, $C_{29}H_{50}O$), 396 (M^+ -H_2O), 381 (M^+ - CH_3, - H_2O), 329 (M^+ - C_5H_7, - H_2O), 273 (M^+-side chain), 255 (M^+ - side chain -H_2O), 231 [M^+-side chain -42 (ring D fragment)], 213 (231-H_2O).

2: ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.69, 0.80, 0.83, 0.84, 0.92, 1.02, 3.52, 5.01 (114, dd, J = 8.6, 16Hz, H-23), 5.15 (1H, dd, J = 8.6, 16Hz, H-22), 5.34; ^{13}C-NMR (see Table 1); m/z 412 (M^+, $C_{24}H_{48}O$), 329, 255, 231, 213.



1 = R = H, R' = $-(CH_2)_2-CH(C_2H_5)-CH(CH_3)_2$

2 = R = H, R' = $-CH=CH-CH(C_2H_5)-CH(CH_3)_2$

3 = R = Glc., R' = $-(CH_2)_2-CH(C_2-H_5)-CH(CH_3)_2$

3: ^{1}H-NMR ($CDCl_3$- CD_3OD, 400 MHz) δ 0.53 (3H, s, H-18), 0.66 (3H, d, J = 8Hz, H-27), 0.68 (3H, d, J = 8Hz, H-26), 0.70 (3H, t, J = 7.4, H-29), 0.79 (3H, d, J = 7.2 Hz, H-21), 0.86 (3H, s, H-19), 3.43 (1H, m, H-3), 4.25 (1H, d, J = 7.6 Hz, H-1'), 5.21 (1H, br, m, H-6); ^{13}C-NMR (see Table 2); m/z 576 (M^+, $C_{35}H_{60}O_6$), 414 (M^+ - sugar), 396, 329, 255, 213.

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